Claims

We claim:

1. A construct for generating a specific oligonucleotide within a cell, which construct comprises a nucleotide sequence from which the transcript is the specific oligonucleotide, said nucleotide sequence being flanked in the 5′ direction by a stabilizing region and in the 3′ direction by a termination sequence, and a promoter, which initiates transcription by RNA polymerase III, and which promoter is in the 5′ direction from the stabilizing region.

2. An oligonucleotide generator, comprising from 5′ to 3′:

(a) an U6-type RNA polymerase III promoter;

(b) a specific nucleotide sequence from which a specific oligonucleotide can be transcribed; and

(c) a termination sequence;

wherein the components of the oligonucleotide generator are operably linked; and wherein the oligonucleotide generator is capable of being transcribed by RNA polymerase III to produce a transcript comprising the specific oligonucleotide.

3. The oligonucleotide generator of claim 2, further comprising:

a stabilizing region from which a first hairpin-forming sequence can be transcribed;

wherein the stabilizing region is operably linked and positioned between the U6-type RNA polymerase III promoter and the specific nucleotide sequence; and wherein the oligonucleotide generator is capable of being transcribed by RNA polymerase III to produce a transcript

comprising from 5′ to 3′ the first hairpin-forming sequence and the specific oligonucleotide.

4. The oligonucleotide generator of claim 3, further comprising

a capping segment;

wherein the capping segment is operably linked and positioned between the stabilizing region and the specific nucleotide sequence; and

wherein the oligonucleotide generator is capable of being transcribed by RNA polymerase III to produce a transcript comprising from 5′ to 3′ the first hairpin-forming sequence, a capping segment of AUAUCC or AUAUAC, and the specific oligonucleotide.

5. The oligonucleotide generator of claim 4, wherein the first hairpin-forming sequence of the transcript consist of the first 20 nucleotides of the naturally-occurring human U6 transcript.

6. The oligonucleotide generator of claim 4, wherein the capping segment of the RNA transcript is AUAUAC.

7. The oligonucleotide generator of claim 2, wherein the U6-type RNA polymerase III promoter is selected from the group consisting of the U6 promoter, the 7SK promoter, the H1 RNA gene promoter, the plant U3 snRNA gene promoter, the MRP gene promoter, and recombinant promoters thereof, which recombinant promoters are capable of initiating transcription by RNA polymerase III from a position upstream of the transcribed DNA.

8. The oligonucleotide generator of claim 7, wherein the U6-type RNA polymerase III promoter is the human U6 promoter.

9. The oligonucleotide generator of claim 2, wherein the specific oligonucleotide is selected from the group consisting of antisense, triplex-forming, ribozyme oligonucleotides, and combinations thereof.

10. The oligonucleotide generator of claim 9, wherein the specific oligonucleotide is an antisense oligonucleotide.

11. The oligonucleotide generator of claim 9, wherein the specific oligonucleotide is a triplex-forming oligonucleotide.

12. The oligonucleotide generator of claim 9, wherein the specific oligonucleotide is a ribozyme oligonucleotide.

13. The oligonucleotide generator of claim 3, further comprising:
a 3′ tail from which a second hairpin-forming sequence can be transcribed;
wherein the 3′ tail is operably linked and positioned between the specific nucleotide sequence and the termination sequence; and
wherein the oligonucleotide generator is capable of being transcribed by RNA polymerase III to produce a transcript comprising from 5′ to 3′ the first hairpin-forming sequence, the specific oligonucleotide, and the second hairpin-forming sequence.

14. The oligonucleotide generator of claim 3, further comprising:

a 3′ tail from which a lariat-forming sequence can be transcribed;

wherein the 3′ tail is operably linked and positioned between the specific nucleotide sequence and the termination sequence;

wherein the oligonucleotide generator is capable of being transcribed by RNA polymerase III to produce a transcript comprising from 5′ to 3′ the first hairpin-forming sequence, the specific oligonucleotide, and the lariat-forming sequence; and

wherein the transcript is predicted to from a stable lariat structure by Watson-Crick base pairing between the nucleotides of the first hairpin-forming region and the lariat-forming region.

15. The oligonucleotide generator of claim 2, further comprising:

a 5′ tail from which a first lariat-forming sequence can be transcribed; and

a 3′ tail from which a second lariat-forming sequence can be transcribed;

wherein the 5′ tail is operably linked and positioned between the U6-type RNA polymerase III promoter and the specific nucleotide sequence;

wherein the 3′ tail is operably linked and positioned between the specific nucleotide sequence and the termination sequence;

wherein the oligonucleotide generator is capable of being transcribed by RNA polymerase III to produce a transcript comprising from 5′ to 3′ the first lariat-forming sequence, the specific oligonucleotide, and the second lariat-forming sequence; and

wherein the transcript is predicted to from a stable lariat structure by Watson-Crick base pairing between the nucleotides of the first lariat-forming region and the second lariat-forming region.

16. The oligonucleotide generator of claim 4, further comprising two oligonucleotides operably linked and position of either side of the termination sequence such that the first 20 nucleotides downstream of the specific nucleotide sequence on the sense strand is identical to the final 20 nucleotide of the 3′ portion of the human U6 gene:

5′ GTCCTAGGCTTTTGCACTTTT 3′;

wherein the U6-type RNA polymerase III promoter is the U6 promoter; and

wherein the first hairpin-forming sequence and the capping segment of the transcript consist of the first 25 nucleotides of the naturally-occurring human U6 transcript.

17. The oligonucleotide generator of claim 16, wherein the sense strand of the specific nucleotide sequence is U6ON:

5′ TCGACTCCTCTTCCTCCTCCACCTCCTCCTCCCATGCA 3′.

18. The oligonucleotide generator of claim 2, further comprising a viral vector capable of inducing integration of the oligonucleotide into a chromosome of a target cell.

19. A method for generating oligonucleotides intracellularly, comprising administering an oligonucleotide generator of claim 2, in a form that permits entry of the oligonucleotides into a target cell.

20. A method for continuously generating oligonucleotides intracellularly, comprising administering an oligonucleotide generator of claim 18 in a form that permits entry of the oligonucleotides into a cell.

21. A generator vector, comprising from 5′ to 3′:

(a) a U6-type promoter;

(b) a stabilizing region from which a hairpin-forming sequence can be transcribed; and

(c) a termination sequence;

wherein the components of the generator vector are operably linked.

22. The generator vector of claim 21, further comprising from 5′ to 3′:

a first restriction enzyme site; and

a second restriction enzyme site;

wherein the first and second restriction enzyme sites are operably linked and positioned between the stabilizing region and the termination sequence.

23. The generator vector of claim 22, further comprising

a capping segment selected from the group consisting of ATATCC and ATATAC;

wherein the capping segment is operably linked and positioned between the stabilizing region and the first restriction enzyme site.

24. The generator vector of claim 23, wherein the U6-type promoter is the human U6 promoter; wherein the hairpin-forming sequence of the transcript

consist of the first 20 nucleotides of the naturally-occurring human U6 transcript; and wherein the capping segment is ATATCC.

25. The generator vector of claim 24, wherein the first restriction enzyme site is an *Xho*I site; and
wherein the second restriction enzyme site is an *Nsi*I site.

26. The generator vector of claim 24, further comprising two oligonucleotides operably linked and position of either side of the termination sequence such that the first 20 nucleotides downstream of the second restriction enzyme site on the sense strand is identical to the final 20 nucleotide of the 3′ portion of the human U6 gene:

5′ GTCCTAGGCTTTTGCACTTTT 3′.

27. A method of measuring triplex formation, which method comprises:

(a) attaching a single-stranded nucleic acid to a solid support;

(b) contacting the solid support with a fluid comprising a labeled double-stranded probe;

(c) separating the unbound probe from the solid support; and

(d) quantifying the amount of labeled double-stranded probe bound to the solid support.

28. A method of measuring triplex blotting, which method comprises:

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(a) attaching a double-stranded nucleic acid to a solid support;

(b) contacting the solid support with a fluid comprising a labeled single-stranded probe;

(c) separating the unbound probe from the solid support; and

(d) quantifying the amount of labeled single-stranded probe bound to the solid support.

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